Appendix 1

Swiss HIV Cohort Study Members

This study has been financed in the framework of the Swiss HIV Cohort Study, supported by the Swiss National Science Foundation (grant # 33CS30_134277 and SHCS-grant #671). The members of the Swiss HIV Cohort Study are: Barth J, Battegay M, Bernasconi E, Böni J, Bucher HC, Burton-Jeangros C, Calmy A, Cavassini M, Cellerai C, Egger M, Elzi L, Fehr J, Fellay J, Flepp M, Francioli P (President of the SHCS), Furrer H (Chairman of the Clinical and Laboratory Committee), Fux CA, Gorgievski M, Günthard H (Chairman of the Scientific Board), Haerry D (deputy of "Positive Council"), Hasse B, Hirsch HH, Hirschel B, Hösli I, Kahlert C, Kaiser L, Keiser O, Kind C, Klimkait T, Kovari H, Ledergerber B, Martinetti G, Martinez de Tejada B, Metzner K, Müller N, Nadal D, Pantaleo G, Rauch A, Regenass S, Rickenbach M (Head of Data Center), Rudin C (Chairman of the Mother & Child Substudy), Schmid P, Schultze D, Schöni-Affolter F, Schüpbach J, Speck R, Taffé P, Tarr P, Telenti A, Trkola A, Vernazza P, Weber R, Yerly S.

Appendix 2

SPARTAC Trial Investigators

Trial Steering Committee (TSC): *Independent Members:* A Breckenridge (Chair), P Clayden, C Conlon, F Conradie, J Kaldor*, F Maggiolo, F Ssali. *Country Principal Investigators:* DA Cooper, P Kaleebu, G Ramjee, M Schechter, G Tambussi, J Weber. Trial Physician: S Fidler. Trial Statistician: A Babiker. Data and Safety Monitoring Committee (DSMC): T Peto (Chair) A McLaren (in memoriam), V Beral, G Chene, J Hakim.Co-ordinating Trial Centre: MRC Clinical Trials Unit, London (A Babiker, K Porter, M Thomason, F Ewings, M Gabriel, D Johnson, K Thompson, A Cursley*, K Donegan*, E Fossey*, P Kelleher*, K Lee*, B Murphy*, D Nock*). Central Immunology Laboratories and Repositories: The Peter Medawar Building for Pathogen Research, University of Oxford, UK (R Phillips, J Frater, L Ohm Laursen*, N Robinson, P Goulder, H Brown). Central Virology Laboratories and Repositories: Jefferiss Trust Laboratories, Imperial College, London, UK (M McClure, D Bonsall*, O Erlwein*, A Helander*, S Kaye, M Robinson, L Cook*, G Adcock*, P Ahmed*). Clinical Endpoint Review Committee: N Paton, S Fidler. Investigators and Staff at Participating Sites: *Australia:* St Vincent's Hospital, Sydney (A Kelleher), Northside Clinic, Melbourne (R Moore), East Sydney Doctors, Sydney, (R McFarlane), Prahran Market Clinic, Melbourne (N Roth), Taylor Square Private Clinic, Sydney (R Finlayson), The Centre Clinic, Melbourne (B Kiem Tee), Sexual Health Centre, Melbourne (T Read), AIDS Medical Unit, Brisbane (M Kelly), Burwood Rd Practice, Sydney (N Doong) Holdsworth House Medical Practice, Sydney (M Bloch) Aids Research Initiative, Sydney (C Workman). *Coordinating centre in Australia*: Kirby Institute University of New South Wales, Sydney (P Grey, DA Cooper, A Kelleher, M Law), *Brazil*: Projeto Praça Onze, Hospital Escola São Francisco de Assis, Universidade federal do Rio de Janeiro, Rio de Janeiro (M Schechter, P Gama, M Mercon*, M Barbosa de Souza, C Beppu Yoshida, JR Grangeiro da Silva, A Sampaio Amaral, D Fernandes de Aguiar, M de Fátima Melo, R Quaresma Garrido), *Italy:* Ospedale San Raffaele, Milan (G Tambussi, S Nozza, M Pogliaghi, S Chiappetta, L Della Torre, E Gasparotto,), Ospedale Lazzaro Spallanzani, Roma (G D'Offizi, C Vlassi, A Corpolongo), *South Africa: Cape Town*: Desmond Tutu HIV Centre, Institute of Infectious Diseases, Cape Town (R Wood, J Pitt, C Orrell, F Cilliers, R Croxford, K Middelkoop, LG Bekker, C Heiberg, J Aploon, N Killa, E Fielder, T Buhler), *Johannesburg*: The Wits Reproductive Health and HIV Institute, University of Witswatersrand, Hillbrow Health Precinct, Johannesburg. (H Rees, F Venter, T Palanee), Contract Laboratory Services, Johannesburg Hospital, Johannesburg (W Stevens, C Ingram, M Majam, M Papathanasopoulos), *Kwazulu-Natal*: HIV Prevention Unit, Medical Research Council, Durban (G Ramjee, S Gappoo, J Moodley, A Premrajh, L Zako), *Uganda:* MRC/Uganda Virus Research Institute, Entebbe (H Grosskurth, A Kamali, P Kaleebu, U Bahemuka, J Mugisha*, H F Njaj*), *Spain:* Hospital Clinic-IDIBAPS, University of Barcelona, Barcelona (JM Miro, M López-Dieguez*, C Manzardo, JA Arnaiz, T Pumarola, M Plana, M Tuset, MC Ligero, MT García, T Gallart, JM Gatell), *UK and Ireland:* Royal Sussex County Hospital, Brighton (M Fisher, K Hobbs, N Perry, D Pao, D Maitland, L Heald), St James's Hospital, Dublin (F Mulcahy, G Courtney, S O'Dea, D Reidy), Regional Infectious Diseases Unit, Western General Hospital and Genitourinary Dept, Royal Infirmary of Edinburgh, Edinburgh (C Leen, G Scott, L Ellis, S Morris, P Simmonds), Chelsea and Westminster Hospital, London (B Gazzard, D Hawkins, C Higgs), Homerton Hospital, London (J Anderson, S Mguni), Mortimer Market Centre, London (I Williams, N De Esteban, P Pellegrino, A Arenas-Pinto, D Cornforth*, J Turner*) North Middlesex Hospital (J Ainsworth, A Waters), Royal Free Hospital, London (M Johnson, S Kinloch, A Carroll, P Byrne, Z Cuthbertson), Barts & the London NHS Trust, London (C Orkin, J Hand, C De Souza), St Mary's Hospital, London (J Weber, S Fidler, E Hamlyn, E Thomson*, J Fox*, K Legg, S Mullaney*, A Winston, S Wilson, P Ambrose), Birmingham Heartlands Hospital, Birmingham (S Taylor, G Gilleran). Imperial College Trial & DSMC Secretariat: S Keeling, A Becker. Imperial College DSMC Secretariat: C Boocock. Funding: SPARTAC was funded by a grant from the Wellcome Trust (069598/Z/02/Z). Abbott Laboratories donated Kaletra/Aluvia (lopinavir and low-dose ritonavir) for the African sites. * Left the study team before the trial ended

Supplementary Methods

Immunohistochemisty

Endogenous peroxidase activity was quenched by two 5 minute incubations in 3% hydrogen peroxide (Sigma-Aldrich, UK) in water followed by 15 minutes in 0.13% sodium azide (Sigma-Aldrich). Slides were blocked with 0.5% blocking reagent (Perkin Elmer, MA USA) in PBS for 30 minutes.

For immunofluorescent staining, slides were stained at 4^{o}C overnight in the Antibody Amplifier (ProHisto, SC USA) with anti-MDR-1 (5A12.2, mouse IgG2b) (Merck Millipore, UK) at 1:1000 dilution. After washing slides were incubated with a 1:100 dilution of peroxidase-conjugated donkey anti-mouse IgG secondary (Jackson Research Laboratories, PA USA) at 37^{o}C for 30 minutes, washed and incubated with TSA-plus Cy5 reagent (Perkin Elmer, MA USA). Slides were then re-blocked with hydrogen peroxide (1x 5 minutes), sodium azide, and 0.5% blocking reagent and incubated with anti-CD3 (F7.2.38, mouse IgG1) (Dako, UK) at 1:200 dilution and anti-CD8 (rabbit polyclonal) (Abcam, UK) at 1:1000 dilution at 37^{o}C for 30 minutes. After washing slides were incubated with peroxidase-conjugated donkey anti-rabbit IgG secondary (Jackson Research Laboratories, PA USA) and then TSA-plus Cy3 reagent (Perkin Elmer, MA USA) as described above to detect CD8. Slides were then reblocked with hydrogen peroxide, and sodium azide and stained with peroxidase-conjugated goat anti-mouse IgG1 secondary (Invitrogen, UK) and TSA-plus FITC reagent (Perkin Elmer, MA USA) to detect CD3. Slides were mounted with Prolong Gold with DAPI (Invitrogen, UK). and imaged on an FluoView FV1000 confocal microscope

(Olympus, Japan). Isotype controls and controls for peroxidase blocking were included in all experiments.

For lipopolysacharide (LPS) staining, samples were processed as above except that R-Buffer A was used and sections were stained with mouse anti-LPS core (WN1 222-5, Hycult Biotech, The Netherlands) at 1:50 dilution at room temperature for 30 minutes. After washing slides were incubated with N-Histofine Simple Stain MAX PO (M) (Nichirei, Japan) at room temperature for 30 minutes. Staining was detected with ImmPACT DAB peroxidase substrate and sections counterstained with Hematoxylin QS (both Vector Labs, UK).

Image Analysis

Images were analysed with CellProfiler 2.0 and CellProfiler Analyst 2.0 software (www.cellprofiler.org; Broad Institute, MA USA) and scored as previously described[24]. Briefly, for the identification of CD161++/MAIT cells objects were identified in CellProfiler based on the DAPI stain. For the identification of LPS+ cells, colours were unmixed and objects identified based on the hematoxylin image. The "Classifier" function in CellProfiler Analyst allows computer based automatic recognition of complex phenotypes by machine learning. For identification of CD161++/MAIT cells, cells were identified as CD3+CD8-MDR1-, CD3+CD8+MDR1-, CD3+CD8-MDR1+, CD3+CD8+MDR1high, CD3+CD8+MDR1lo or none of the above. The accuracy of object identification and classification was assessed by manually counting a subset of randomly selected images. The results obtained with CellProfiler Analyst correlated strongly with the manual count for total CD3+ cells (R^2=0.74), CD3+CD8+ cells (R^2=0.96), CD3+CD8+MDR1++ cells (R^2=0.88), and the proportion of CD3+CD8+ cells expressing MDR1++ (R^2=0.60).

Surface area analysed, excluding the intestinal lumen, was calculated in ImageJ version 10.2 (National Institutes of Mental Health, MD, USA).

Table S1

Clinical characteristics of HIV+ cohorts

	Early HIV+	**Chronic HIV+ (PBMC)**	**Chronic HIV+ (Whole blood)**
n	35	13	10
Gender Male Female	 35 0	 10 3	Unknown
Age Median (IQR)	34 (27, 39)	39 (31, 47)	Unknown
CD4 count (cells/ml) Median (IQR)	603 (207, 326)	250 (475, 774)	470 (450, 667)
Viral load (log10 cpm) Median (IQR)	4.73 (3.89, 5.19)	4.22 (3.99, 4.94)	4.48 (3.01, 4.95)
Estimated time since diagnosis Median (IQR)	61 (39, 86) weeks*	48 (7.5, 90) months	15 (12, 72) months

*estimated time from seroconversion

Table S2

Clinical characteristics of treatment cohort from SHCS

	Treatment time point		
	Pre treatment	**1 year post treatment**	**2 years post treatment**
n	29	29	29
Gender			
Male	26		
Female	3		
Age			
Median (IQR)	40 (34.5, 44.5)		
Risk Group			
MSM	18		
Het	9		
IDU	1		
Unknown	1		
CD4 count (cells/ml)			
Median (IQR)	202 (187, 299.5)	414 (319.5, 568)	503 (403, 602)
Viral load (log10 cpm)			
Median (IQR)	4.64 (4.09, 4.99)	0 (0, 0)	0 (0, 0)
Time since diagnosis (months)			
Median (IQR)	10 (2, 92)		

Table S3

Clinical characteristics of HIV+ patients who underwent colon biopsy

	HIV+
n	12
Gender Male Female	 12 0
Age Median (IQR)	 40 (31, 41)
CD4 count (cells/ml) Median (IQR)	 577 (474, 616)
Viral load (log10 RNA copies/ml) Median (IQR)	 3.64 (1.60, 5.29)
Time since diagnosis (months) Median (IQR)	 37 (23, 67)

Supplemental Figure legends

Supplementary figure 1. Correlation between IFNγ and IL17A production by CD161++CD8+T-cells in response to stimulation with *E. coli*. (a) For each donor in figure 1 the percentage of CD161++CD8+T-cells producing IFNγ or IL17A is shown. (b) There was no correlation between the percentage of CD161++CD8+ T cells producing IFNγ and the percentage producing IL17A.

Supplementary figure 2. Loss of CD161++/MAIT- and Tc17 cells in HIV infection. (a,b) Analysis of freshly isolated whole blood from healthy controls and chronic stage HIV+ patients showing the percentage of the CD8+T-cell population expressing CD161++ (a) and CD161+ (b). (c,d) No correlation was seen between the percentage of CD161++CD8+T-cells and the viral load (c) or the CD4 count (d).

Supplementary figure 3. Differential expression of chemokine receptors by CD8+T-cells in HIV infection. Analysis of freshly isolated whole blood from healthy controls (green triangles) and chronic stage HIV+ patients (blue triangles) for expression of (a,b) CCR6, CCR5 and CXCR4 on CD8+T-cells and (c) CCR6 expression on CD4+T-cells.

Supplementary figure 4. CD161++CD8+T-cells in the colon express high levels of MDR1. Lamina propria mononuclear cells (LPMCs) were analysed by flow cytometry for expression of CD161 and MDR1. (a) A CD161++CD8+T-cell population

was evident in LPMC. (b,c) The highest levels of MDR1 expression (blue) on CD8+T-cells were seen on the CD161++ population (isotype is shown in red).

Supplementary figure 5. The effect of HIV, HAART, and non-specific colitis on T-cell populations in the colon. Sections of colon were stained as in Fig. 3. The total number of (a) CD3+ cells per mm^2 was increased in HIV infection. (b) No difference was seen in the number of CD3+CD8- cells per mm^2. The effect of HAART (c,d) and non-specific colitis (e,f) on the number of (c,e) MDR1++CD8+CD3+ and (d,f) CD8+CD3+ cells in the colon of HIV-infected patients is shown.

Supplementary figure 6. CD161++CD8+T-cell proliferation and apoptosis in response to stimulation with *E. coli*. Correlation of the number of LPS+ lamina propria cells/mm^2 with (a) the percentage of CD3+CD8+ cells expressing MDR1++ and (b) the number of CD3+CD8+MDR1++ cells/mm^2 in the colon of HIV infected patients (n=12). (c,d) PBMCs were stained with CellTrace Violet (CTV) and cultured for 6 days with varying amounts of PFA-fixed *E. coli*. Dose-dependent proliferation was seen in the CD161++CD8+T-cell population alone. (e) Despite *E. coli*-induced proliferation, CD161++CD8+T-cells were specifically lost from the culture; cell survival was defined as the percentage of CD8+T-cells expressing the indicated level of CD161 in the treated compared to the untreated culture. Blocking MR1 with antibody 26.5 (10μg/ml) partially inhibited proliferation (f,h) and loss (g) of CD161++CD8+T-cells. (i,j) PBMCs were stimulated with PFA-fixed *E. coli* and surface expression of (i) phosphatidylserine (PS; expressed as a ratio of %PS+ to the

untreated control) and (j) cell survival. (k) Phosphatidylserine expression and (l) cell survival at 44 hours were also assessed with PBMCs from HIV infected patients. (m,n) Non-specific stimulation of PBMCs from healthy controls with anti-CD2/CD3/CD28 microbeads at the indicated bead-to-cell ratio induced dose-dependent apoptosis in all CD8+ T cells, irrespective of CD161 expression, as assessed by expression of (m) activated caspase 3 at 20 hours and (n) phosphatidylserine at 44 hours.

Supplementary figure 7. Stimulation with *E. coli* causes upregulation of death receptors on CD161++CD8+T-cells. PBMCs were treated for 20 hours with PFA-fixed *E. coli* and expression of (a,b) CD95 (Fas), (c,d) TNFRI, (e,f) TNFRII, (g,h) DR4, (i,j) DR5, and (k,l) Bcl2 assessed. (m,n,o) PBMCs were stimulated with *E. coli* (10 BpC) in the presence of either ant-Fas (1ug/ml), TNFRI/TNFRSF1A Fc chimera (1ug/ml), both anti-Fas and TNFRI/TNFRSF1A Fc chimera, or an isotype control and (m) the expression of (m) activated caspase 3 at 20 hours and (n) phosphatidylserine at 44 hours by CD161++CD8+T-cells determined (both expressed as the percentage positive relative to PBMC treated with *E. coli* alone). (o) Survival of CD161++CD8+T-cells at 44 hours was also determined (defined as the percentage of CD161++CD8+T-cells in the treated compared to the untreated culture).

Supplementary figure 8. Model for loss of CD161++/MAIT-cells from blood in HIV infection. The model shows a schematic of blood, gut, gut lumen and other

tissues (including liver and spleen). CD161++/MAIT-cells and other CD8+T-cells are shown as indicated, and bacteria/bacterial antigen in red. Processes, are annotated with a * if we have evidence for them in this or other studies, and in italics if this evidence is *in vitro*. In A (healthy state) there is natural redistribution of CD161++/MAIT-cells to tissue. Here the processes of proliferation, death and possibly return to the blood maintain a steady state. B shows the impact of HIV, which may occur very early. Here there is bacterial translocation, and this may be associated with further recruitment to gut, and the balance of proliferation and death may shift in favour of death at high levels of antigen. Similar recruitment and activation may occur in other tissues, such as liver and spleen. In C, after HIV treatment, the factors which may limit return to the healthy state are indicated by underlining. These include a failure to fully resolve microbial translocation, leading to ongoing recruitment, activation/death and/or retention, a failure to proliferate, and failure to generate new populations *de novo*.

Supplementary figure 9. Gating strategies. (a) The gating strategy for PMA/ionomycin stimulation of PBMC is shown. The gating for (b) the in vitro infection assay, including (c) how the frequency of infection of each CD161 subset and (d) the contribution of each CD161 subset to the total CD8 infection were calculated, is shown.

Figure S1

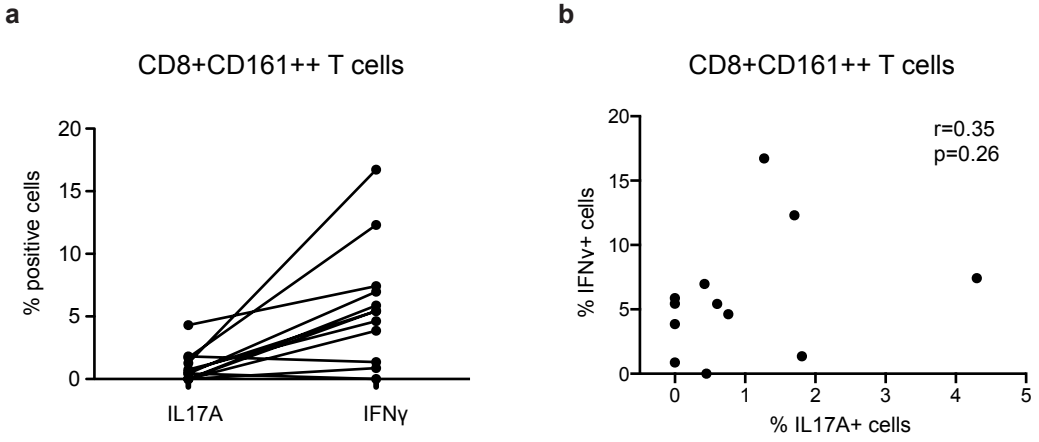
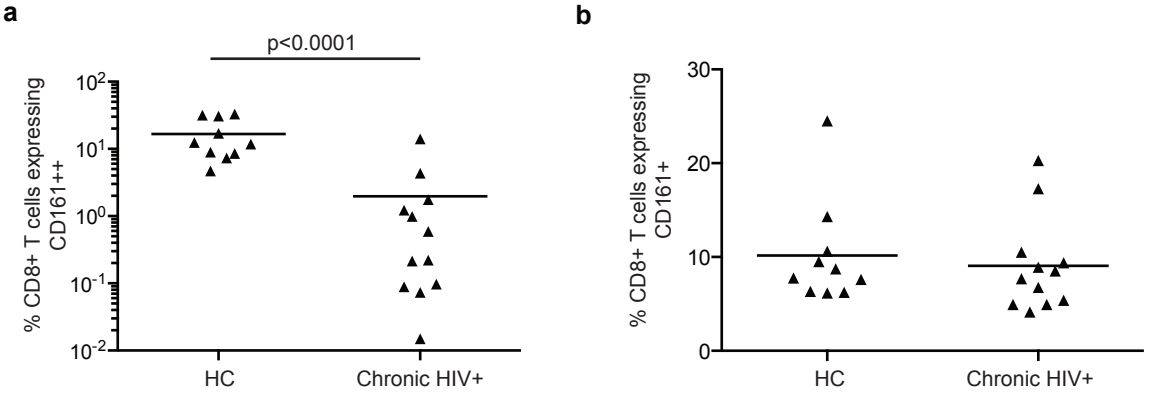

a
CD8+CD161++ T cells
% positive cells
20
15
10
5
0
IL17A
IFNγ
b
CD8+CD161++ T cells
r=0.35
p=0.26
% IFNv+ cells
% IL17A+ cells
0
1
2
3
4
5

Figure S2

Fresh whole blood analysis of CD161 expression on CD8+ T cells



Correlations of CD161++CD8+T cell frequency with clinical data in untreated HIV+ patients

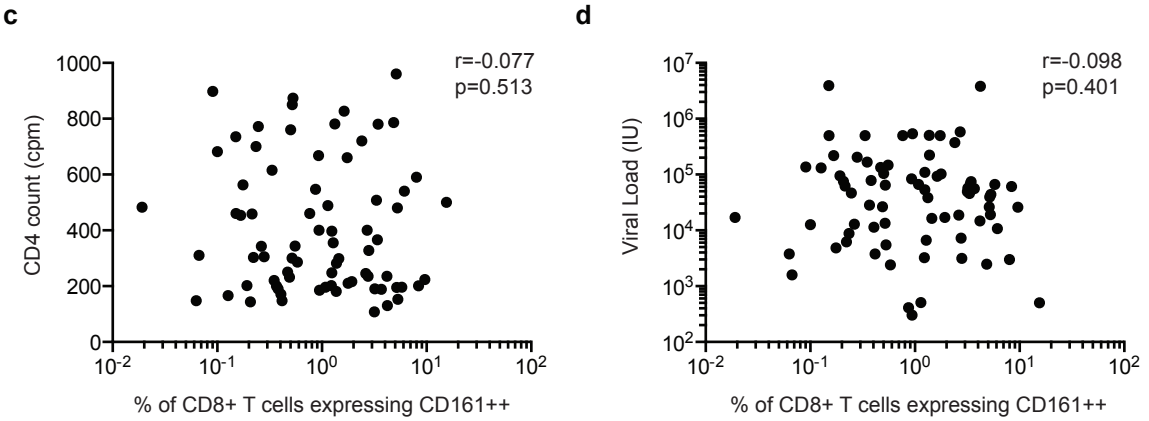


Figure S3

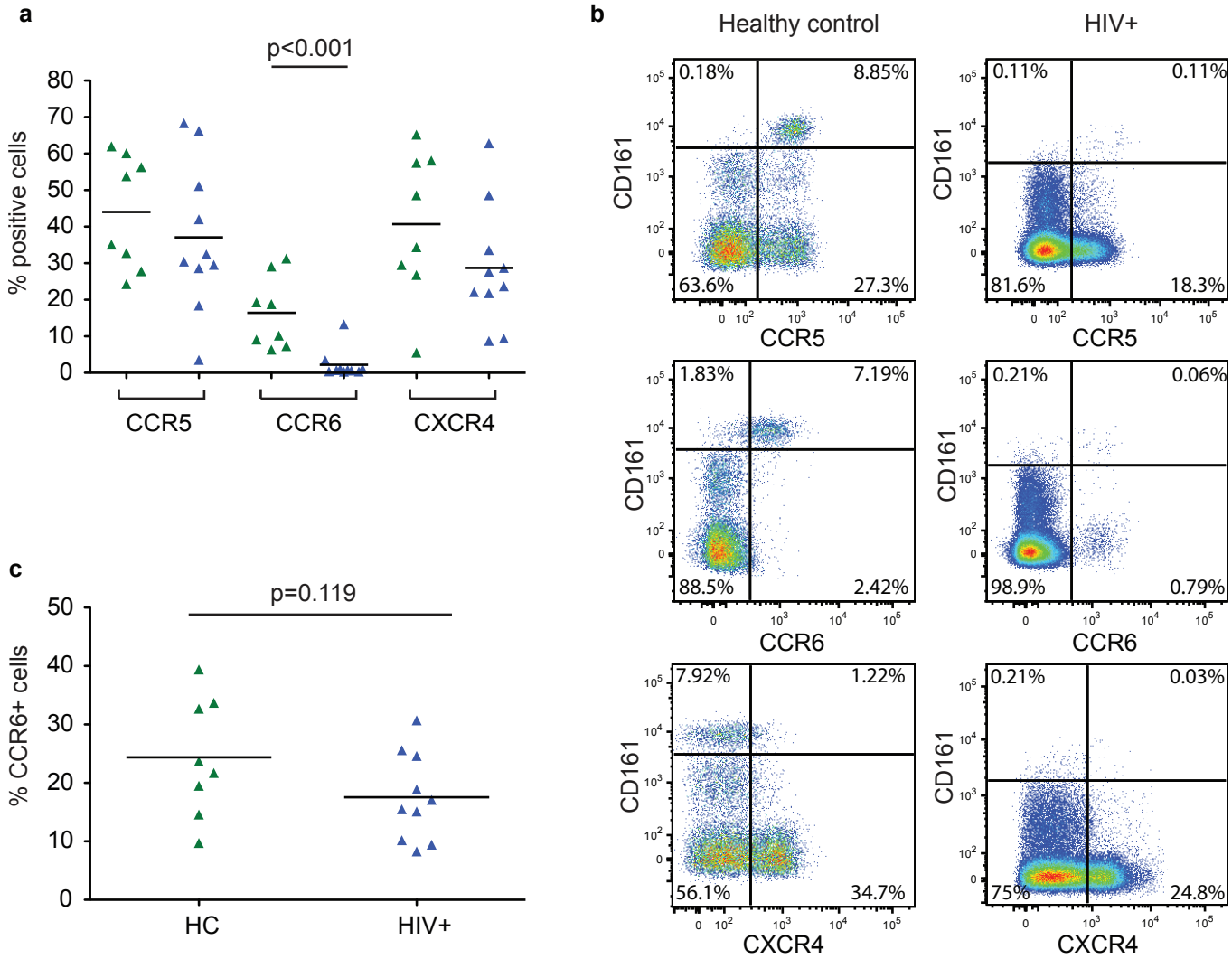

a
p<0.001
% positive cells
80
70
60
50
40
30
20
10
0
CCR5
CCR6
CXCR4
b
Healthy control
HIV+
CD161
CCR5
0.18%
8.85%
63.6%
27.3%
0.11%
0.11%
81.6%
18.3%
CCR6
1.83%
7.19%
88.5%
2.42%
0.21%
0.06%
98.9%
0.79%
CXCR4
7.92%
1.22%
56.1%
34.7%
0.21%
0.03%
75%
24.8%
c
p=0.119
% CCR6+ cells
50
40
30
20
10
0
HC
HIV+

Figure S4

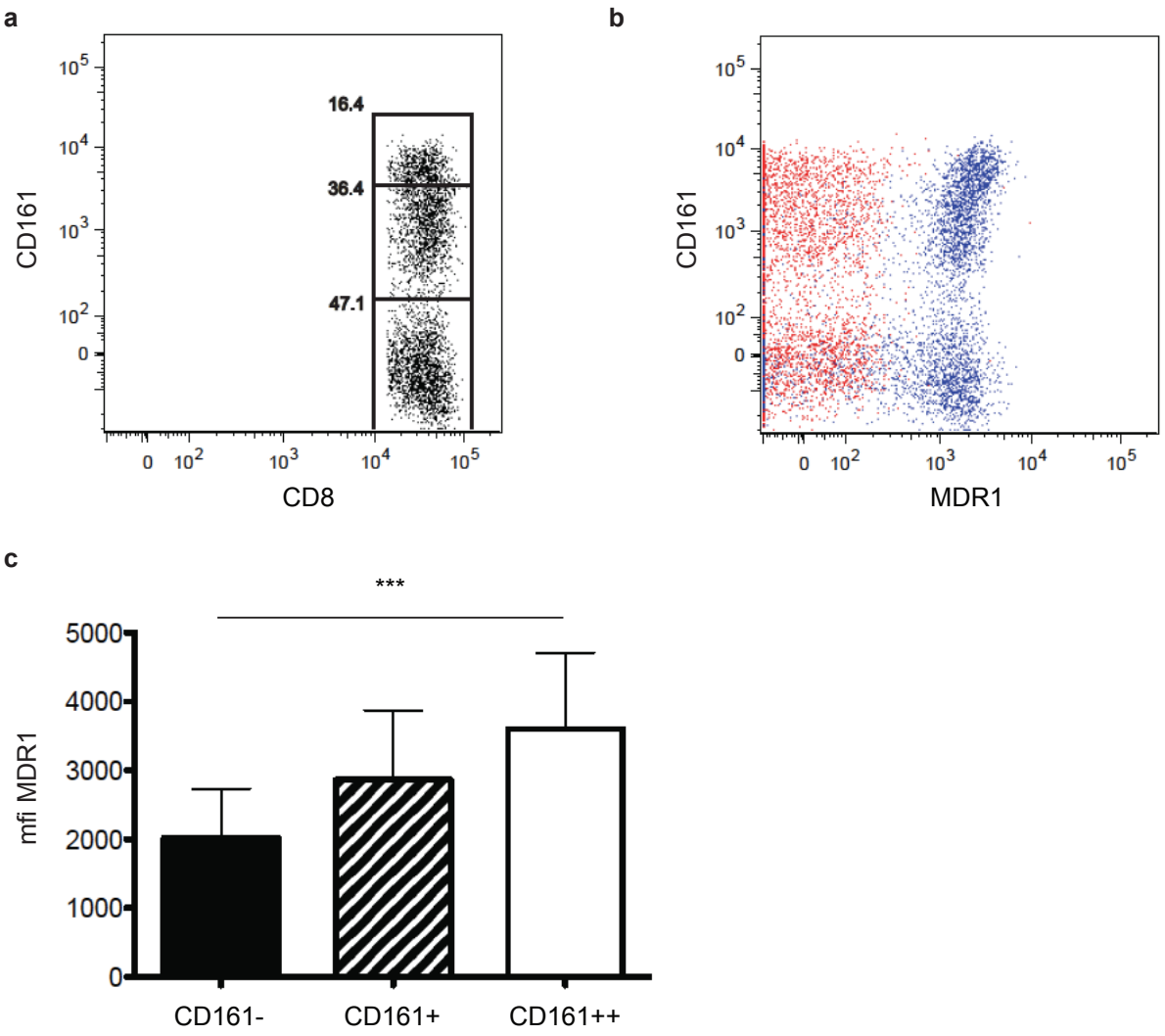


Figure S5

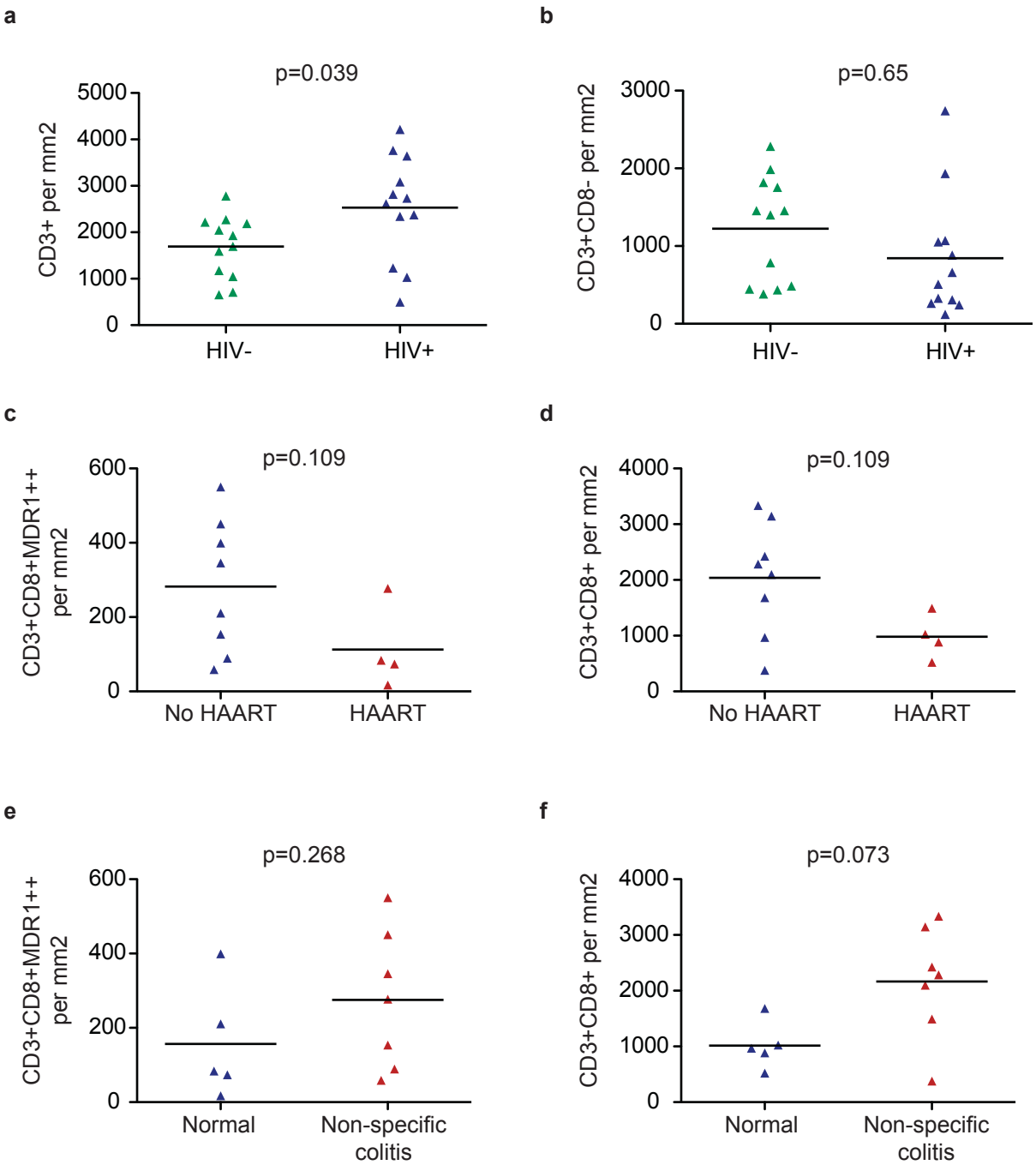

a
p=0.039
CD3+ per mm2
5000
4000
3000
2000
1000
0
HIV-
HIV+
b
p=0.65
CD3+CD8- per mm2
3000
2000
1000
0
HIV-
HIV+
c
p=0.109
CD3+CD8+MDR1++ per mm2
600
400
200
0
No HAART
HAART
d
p=0.109
CD3+CD8+ per mm2
4000
3000
2000
1000
0
No HAART
HAART
e
p=0.268
CD3+CD8+MDR1++ per mm2
600
400
200
0
Normal
Non-specific colitis
f
p=0.073
CD3+CD8+ per mm2
4000
3000
2000
1000
0
Normal
Non-specific colitis

Figure S6

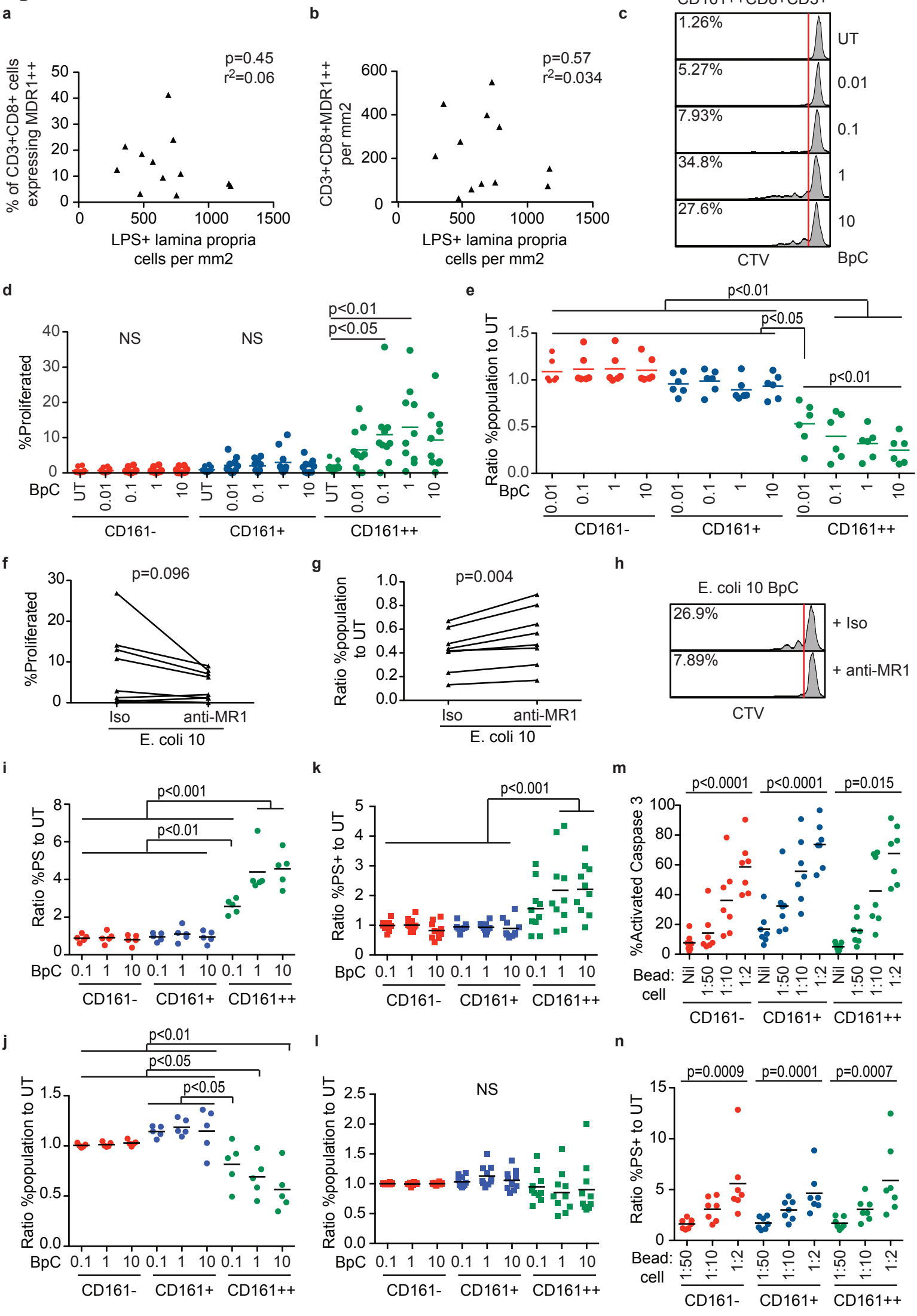
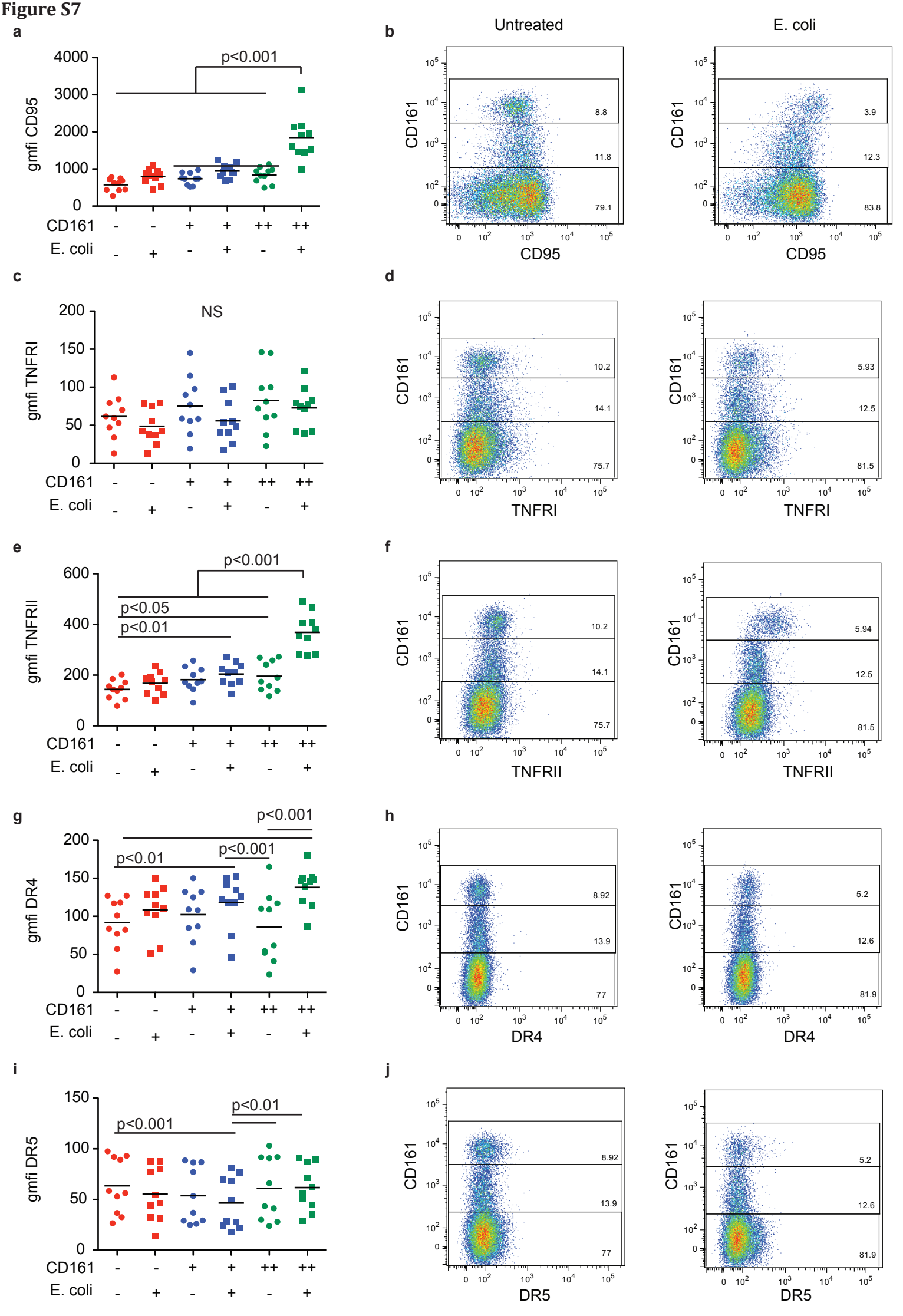

a
% of CD3+CD8+ cells expressing MDR1++
p=0.45
r2=0.06
LPS+ lamina propria cells per mm2
b
CD3+CD8+MDR1++ per mm2
p=0.57
r2=0.034
LPS+ lamina propria cells per mm2
c
CD161++CD8+CD3+
1.26%
5.27%
7.93%
34.8%
27.6%
UT
0.01
0.1
1
10
CTV
BpC
d
%Proliferated
NS
NS
p<0.01
p<0.05
BpC
CD161-
CD161+
CD161++
e
Ratio %population to UT
p<0.01
p<0.05
p<0.01
BpC
CD161-
CD161+
CD161++
f
%Proliferated
p=0.096
Iso
anti-MR1
E. coli 10
g
Ratio %population to UT
p=0.004
Iso
anti-MR1
E. coli 10
h
E. coli 10 BpC
26.9%
7.89%
+ Iso
+ anti-MR1
CTV
i
Ratio %PS to UT
p<0.001
p<0.01
BpC
CD161-
CD161+
CD161++
k
Ratio %PS+ to UT
p<0.001
BpC
CD161-
CD161+
CD161++
m
%Activated Caspase 3
p<0.0001
p<0.0001
p=0.015
Bead: cell
Nil
1:50
1:10
1:2
CD161-
CD161+
CD161++
j
Ratio %population to UT
p<0.01
p<0.05
p<0.05
BpC
CD161-
CD161+
CD161++
l
Ratio %population to UT
NS
BpC
CD161-
CD161+
CD161++
n
Ratio %PS+ to UT
p=0.0009
p=0.0001
p=0.0007
Bead: cell
CD161-
CD161+
CD161++

Figure S7


a
gmfi CD95
p<0.001
CD161
E. coli
b
Untreated
E. coli
CD161
CD95
8.8
11.8
79.1
3.9
12.3
83.8
c
gmfi TNFRI
NS
d
TNFRI
10.2
14.1
75.7
5.93
12.5
81.5
e
gmfi TNFRII
p<0.001
p<0.05
p<0.01
f
TNFRII
10.2
14.1
75.7
5.94
12.5
81.5
g
gmfi DR4
p<0.001
p<0.01
p<0.001
h
DR4
8.92
13.9
77
5.2
12.6
81.9
i
gmfi DR5
p<0.001
p<0.01
j
DR5
8.92
13.9
77
5.2
12.6
81.9

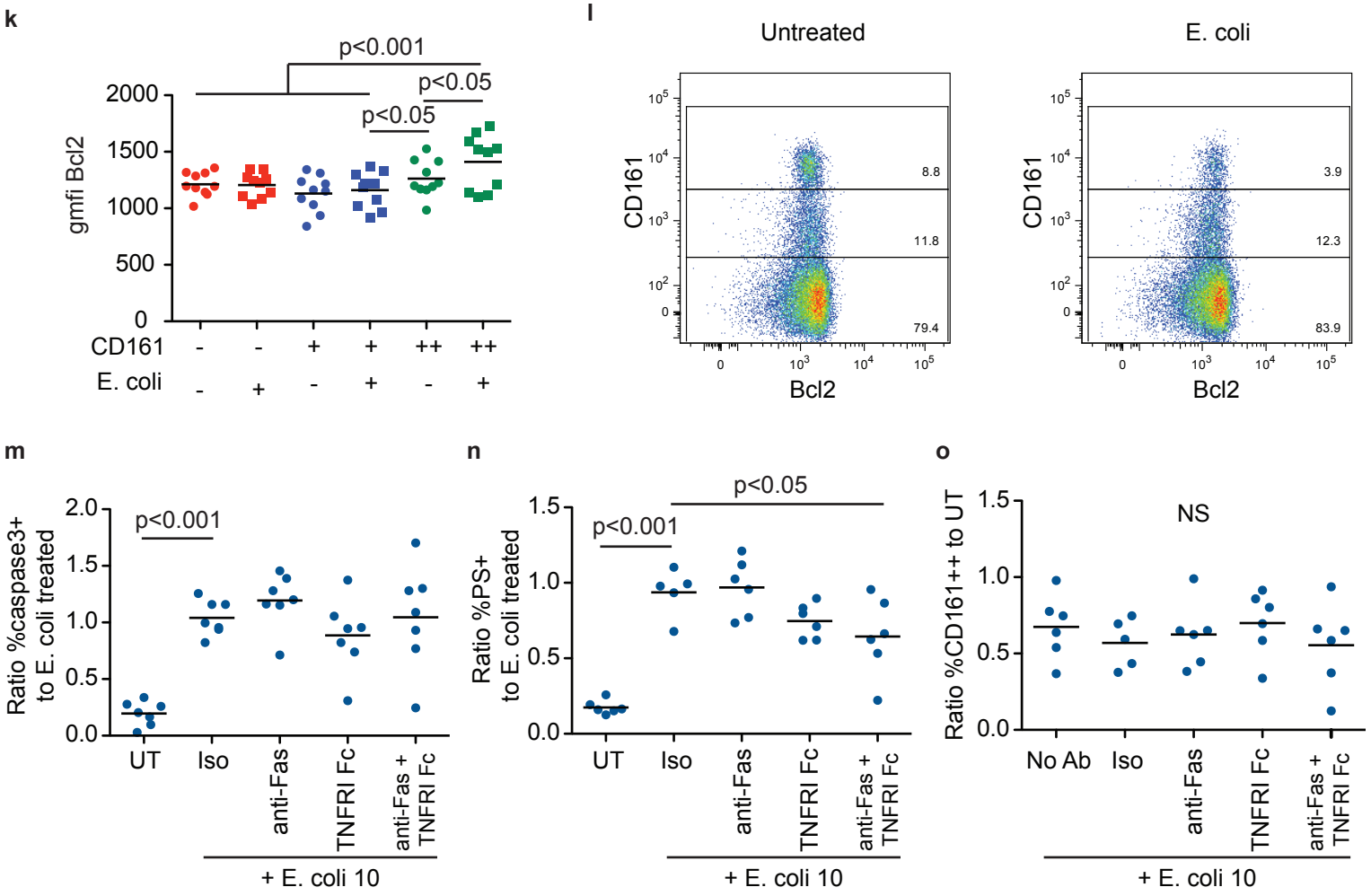

k
p<0.001
p<0.05
p<0.05
gmfi Bcl2
2000
1500
1000
500
0
CD161 - - + + ++ ++
E. coli - + - + - +
l
Untreated
E. coli
CD161
Bcl2
8.8
11.8
79.4
3.9
12.3
83.9
m
Ratio %caspase3+ to E. coli treated
p<0.001
UT
Iso
anti-Fas
TNFRI Fc
anti-Fas + TNFRI Fc
+ E. coli 10
n
Ratio %PS+ to E. coli treated
p<0.05
p<0.001
o
Ratio %CD161++ to UT
NS
No Ab

Figure S8

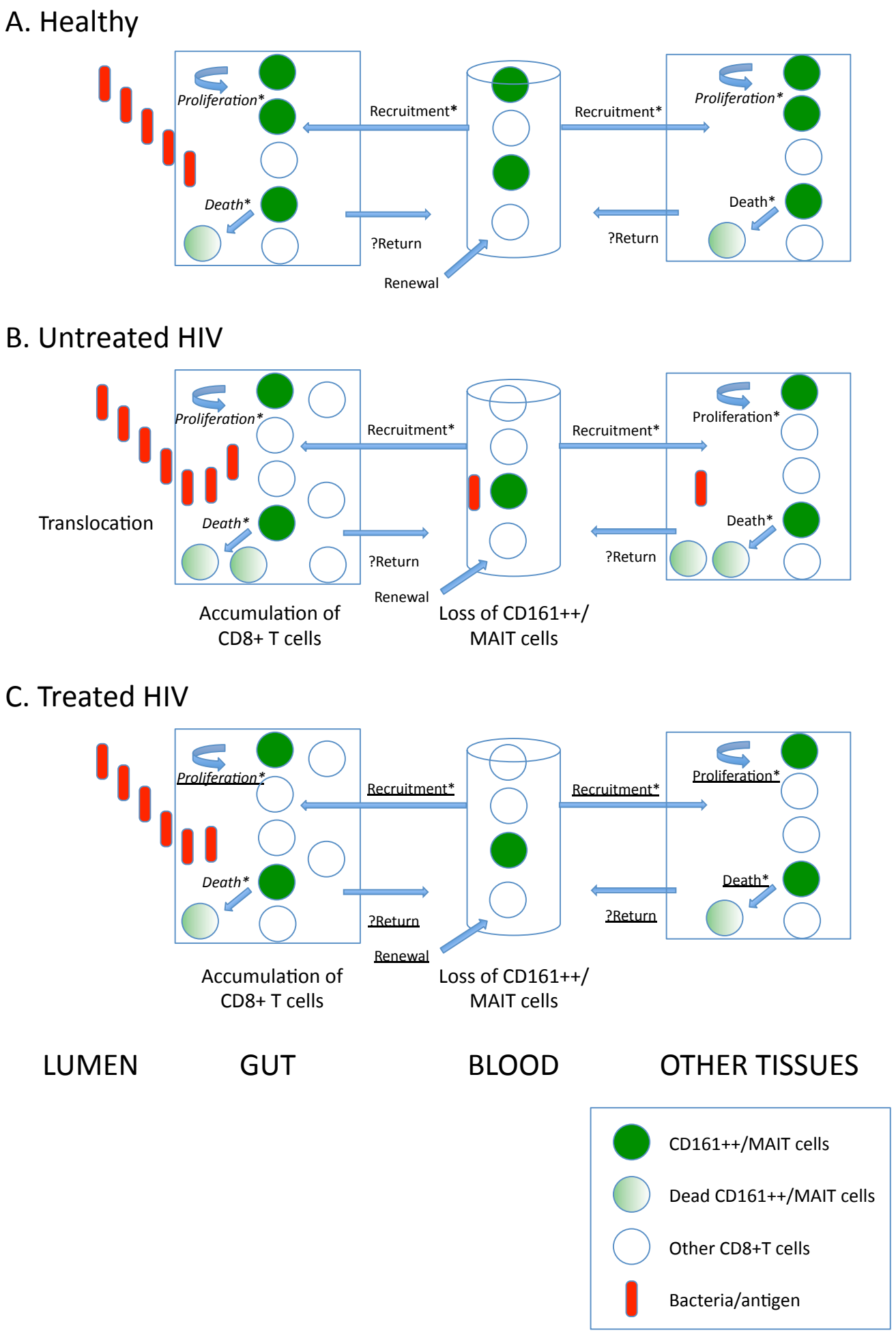
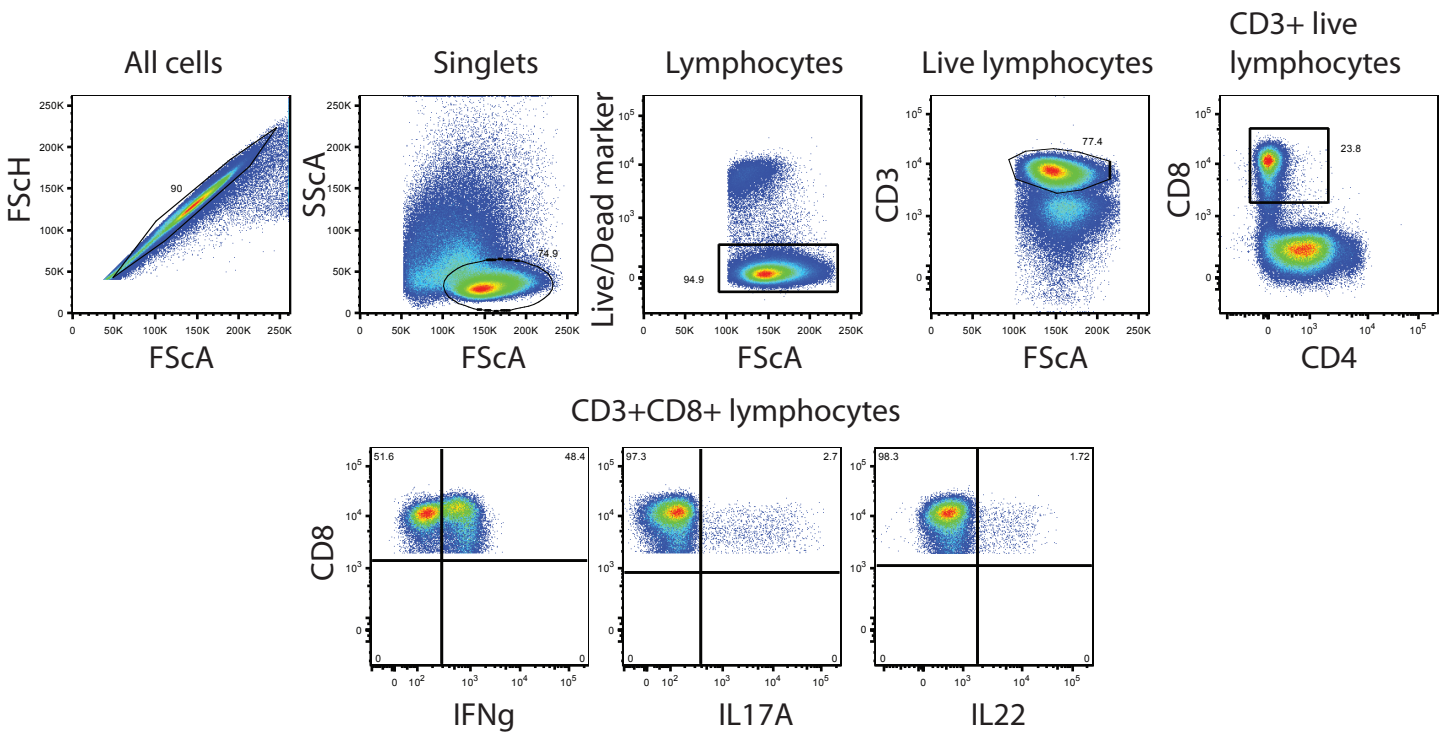
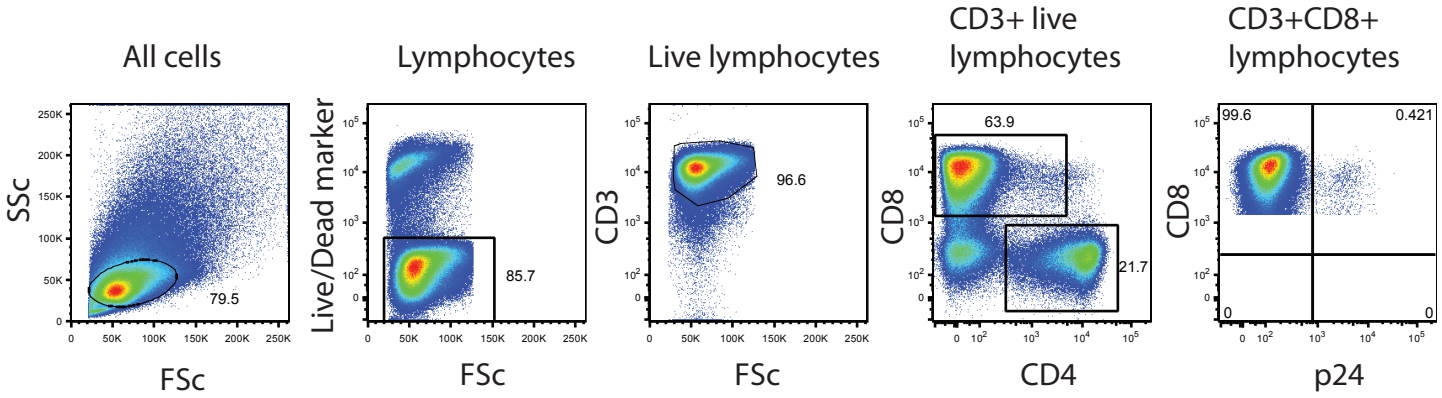

A. Healthy
Proliferation*
Death*
Recruitment*
?Return
Renewal
Recruitment*
?Return
Proliferation*
Death*
B. Untreated HIV
Translocation
Proliferation*
Death*
Recruitment*
?Return
Renewal
Recruitment*
?Return
Proliferation*
Death*
Accumulation of CD8+ T cells
Loss of CD161++/ MAIT cells
C. Treated HIV
Proliferation*
Death*
Recruitment*
?Return
Renewal
Recruitment*
?Return
Proliferation*
Death*
Accumulation of CD8+ T cells
Loss of CD161++/ MAIT cells
LUMEN
GUT
BLOOD
OTHER TISSUES
CD161++/MAIT cells
Dead CD161++/MAIT cells
Other CD8+T cells
Bacteria/antigen

Figure S9

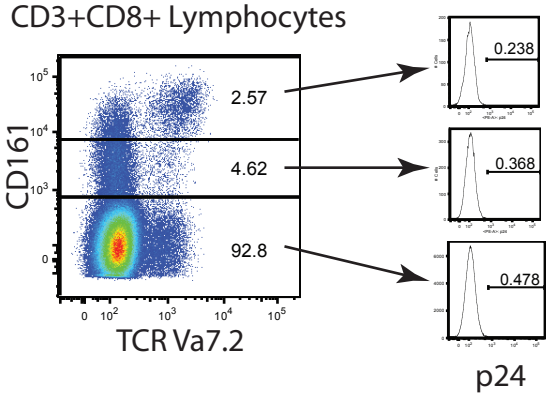
A - gating strategy for PMA/Ionomycin stimulation of PBMC



B - gating strategy for in vitro infection assay



C - gating to calculate the frequency of infection of each CD161 subset



D - gating to calculate the contribution of each CD161subset to the total CD8 infection